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Utility of Specific Locus Systems in Higher Plants to Monitor for Mutagens

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Plants possess biological and operational attributes that have encouraged geneticists to use them extensively in the development of fundamental genetic concepts. Attributes such as regenerative plasticity, high fecundity, cultural adaptability, range of ploidy, economics of culture and maintenance of specific populations, and versatility make plant genetic systems prime candidates with which to monitor the environment for mutagens.

A specific locus (equivalent to a classical Mendelian gene) controls the development of a phenotypic characteristic. It can also mutate to a new allelic form with a consequentially altered phenotypic characteristic and can be separated by crossing over from adjacent loci that govern other specific phenotypic characteristics. Since various plant species have numerous specific loci, one has a rich array of potential systems from which to select.

Specific locus systems in higher plants could be used to assess the mutagenicity of single chemical compounds or combinations of chemical compounds. Depending on the circumstances, seeds and/or seedlings could be used; plants could be grown *in situ* in either containers or plots to assess the immediate environment for one or more mutagens over an extended period. Since plants are eucaryotes, data from such experiments could serve as one more source of information along with that obtained from a battery of other tests used in the tier system.

Introduction

Specific locus systems in higher plants have been used for experiments with chemical and physical mutagens. Generally, the objectives have been to assess methods of treatment and cultural management relative to their application in mutation breeding for crop improvement. However, the purpose of this Workshop is to evaluate the potential of higher plant systems to monitor the environment for mutagens. I should therefore like to emphasize attributes of higher plants as potential systems to monitor for mutagens, describe three specific locus systems which are not covered otherwise in this Workshop program, and define the role that specific locus systems in higher plants can play in assessing the mutagenicity of pesticides, industrial pollutants, etc.

Plant Genetics Relative to Other Fields of Genetics

In *Genetics of Flowering Plants*, Grant (*1*) devotes one chapter to a comparison of the impact of plant genetics with that of molecular, microbial, population, and human genetics in relation to their actual and potential role in modern biology. Grant emphasizes that plant genetics no longer enjoys the dominant position in the overall science of genetics that it once did. In addition to being the organism chosen for Mendel's pioneering experiments which formed the foundations for the science of genetics, plant systems have been used in numerous experiments, the results of which have contributed significantly to the development of fundamental concepts. I would like to view these past accomplishments in the context of what can be gained in terms of understanding the role that plants can play in monitoring environmental quality.

Attributes of Higher Plants as Monitors of Mutagens

Let us consider some attributes of higher plants that have induced geneticists to use them extensively in the past. Which of these attributes are of significant value today in monitoring the environment for mutagens?

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Biological Attributes

Regenerative Plasticity. The numerous modes of reproduction in plants, including sexual and asexual propagation (frequently within the same individual), permit unique populations to be established. For example, controlled crosses can be made readily to obtain desirable heterozygous genotypes that can then be reproduced vegetatively to establish clones for genetic studies. Rick (*2*) discusses this topic in relation to the tomato as a species for genetic studies.

Fecundity. Large numbers of seeds from which offspring can be derived, generally at will, are produced by a single plant and in some cases by a single ovary. For example, a single plant of *Arabidopsis* can yield over 50,000 seeds (*3*), one of *Mimulus cardinalis* over 200,000 seeds (*4*), and one of tobacco over 1,000,000 seeds (*5*).

Cultural Adaptability. Many plant species can be cultured successfully under a wide range of environmental conditions from axenic culture in laboratory test tubes to pot culture either indoors or outdoors to extensive plantings in the field.

Generation Time. The time required to complete a life cycle varies from one month for *Arabidopsis* (*3*) to many years for certain trees. Certain genetic systems do not require a complete life cycle for use in experiments.

Ploidy. A wide range of ploidy levels exists in nature among species of plants; however, ploidy can be manipulated readily within a species. High polyploids are frequently fertile, and haploids can be reproduced asexually. *In vitro* culture techniques offer the potential for developing haploids in sufficient number for experimental purposes (*6*). Certain plants including *Allium, Lilium, Tradescantia, Vicia,* and *Hordeum* have both large chromosomes and few chromosomes which facilitate cytogenetic studies.

Developmental Morphology. The relatively simple ontogeny found in plants permits various chimeras that are not found in other kinds of organisms. Some of these chimeras can be used advantageously in genetic studies (*1*).

Operational Attributes

Economics. Culture of large populations of plants under either field or laboratory conditions can be achieved with relative ease and little expense compared to culture of animal populations of similar size. Facilities required for plant populations are less extensive than those for animal colonies (*7*).

Maintenance of Organisms for Experiments. Seeds, vegetative reproductive structures, and now *in vitro* cultured cells can be kept in long-term storage to circumvent significant investments to maintain test organisms. Storage of large inventories is feasible, making available a ready supply of uniform population for test purposes over a long period.

Versatility. Seeds and certain vegetative reproductive parts can be handled with little regard for time and temperature. One can utilize seeds, for example, to study the interaction of physical and chemical agents as expressed at the genetic level (*7*). Factors such as anoxic atmosphere can be tolerated with relative ease by seeds, whereas the same conditions would be either toxic or lethal to most animals. Populations of plants can be grown at sites far removed from laboratory facilities for relatively long periods of minimum attention to site preparation, maintenance, etc.

Applicability of Experimental Data

The present tiered system approach for testing the mutagenicity of various compounds attests to the problems inherent in extrapolation of data from one system to another. It must be emphasized, however, that the degree of genetic complexity in higher plants is comparable to that which exists in humans. Indeed, both humans and higher plants are eucaryotic organisms, so their chromosome structure and biochemistry may be similar (*8*). Other similarities include DNA content per cell, extent of repetitive DNA, regulation of gene action relative to ontogeny, numbers of genes, etc. From a historical perspective, those fundamental genetic concepts based on results of experiments with plants have been found for the most part to be applicable to animals. Of course, one cannot ignore the problems associated with species differences caused by metabolism, routes of ingestion, metabolic activation, etc.

Definition of Specific Locus

A specific locus is equivalent to a gene in the classical Mendelian sense. As such, a specific locus is defined as a chromosomal region that controls the development of a phenotypic characteristic, that can mutate to a new allele with a consequentially altered phenotypic characteristic, and that is separable by crossing over from adjacent loci governing other specific phenotypic characteristics.

Specific Locus Systems in Higher Plants

Numerous specific loci, according to the definition given above, have been characterized in plant

species. In fact, reference is made to 250 loci associated with the seven linkage groups of barley (*7*), over 1100 genes in the cultivated tomato (*2*), over 300 loci for maize (*9*), 55 genes in *Cucumis (10)*, and 500 loci readily available in peas (*11*). Obviously the scope of this report does not permit complete coverage of the literature dealing with these systems.

Plants such as *Antirrhinum, Oenothera, Zea, Arabidopsis, Tradescantia, Lycopersicon,* and *Hordeum* have been used extensively for mutagenesis experiments involving both chemical and physical mutagens. Frequently the protocol for these experiments is similar, if not identical, to that which would be required to monitor for mutagens.

The Chlorophyll Deficiency System in Barley

The Organism. *Hordeum vulgare* L. emend. Lam., cultivated barley, is a diploid with $2N = 14$ chromosomes that are relatively large and easily identified. The plant is self-fertilized; thus recessive mutations are not lost through outcrossing. Hybridization is accomplished easily which makes genetic studies relatively easy to complete.

The System. Cultivated barley was used quite early in studies of mutation induction by ionizing radiation. Discrete phenotypes involving either a deficiency or lack of chlorophyll were observed frequently in segregating generations following treatment with mutagens. These phenotypes were described and classified by Gustafsson (*12*) in 1940. Numerous gene loci are responsible for these chlorophyll-deficient phenotypes; estimates include 250–300 (*13*), and several hundred (*14*) for the *Albina* phenotype alone. Most of these chlorophyll-deficient mutants behave as monofactorial recessives. Constantin (*15*) recently reviewed this system.

Procedures. The barley system has been used for various types of experiments involving mutagenesis. One involves the treatment of seed with either a single mutagen or a combination of mutagens. When seeds are treated, the M_1 generation plants can be used to assess morphophysiological effects such as degree of reduction in seedling height, survival to maturity, fertility, etc. The M_2 generation is used to assess the degree of genetic effects usually based on the frequency of chlorophyll-deficient phenotypes. This approach measures genetic effects in germinal tissue. It is relatively simple to relate genetic effects induced by a mutagen (pollutant) observed in the M_2 generation to the toxic effects induced by the same mutagen observed in the M_1 generation.

The dormant embryo of barley has up to four leaf primordia and two visibly differentiated axillary buds in addition to the shoot apex. Each plant can develop up to six primary tillers from distinct primordia plus numerous higher order tillers. A mutation induced in a cell of one primary tiller will not be expressed in any other primary tiller. Thus chimeras are to be expected at the M_1 plant level. In addition, from one to four initial cells participate to form a spike; thus chimeras are to be expected within a spike. In fact, chimeras have been observed at the individual anther level. It should be noted that cells of the dormant embryo (dry seeds) are arrested predominantly in G_1 phase of the DNA cycle; however, when soaked seeds are treated the duration of the soaking period will determine the phase of the DNA cycle for the majority of cells. This influences the degree of genetic effects.

Another type of experiment involves the exposure of plants to a mutagen throughout an entire life cycle. Ionizing radiation has frequently been the mutagen of choice in such experiments. In this case, the M_0 plants (the generation of individuals grown in the presence of the mutagen) are grown to maturity and harvested individually. M_1 progeny is grown from each M_0 plant in the absence of the mutagen, and individual M_1 plants are harvested. The M_2 generation is used to determine the frequency of chlorophyll-deficient phenotypes. In this type of experiment, one measures an effect integrated across different developmental stages which might vary considerably in their vulnerability to the mutagen.

Results. Figure 1 shows chlorophyll-deficient mutants per 10^3 M_2 seedlings of Himalaya barley induced by various concentrations of ethylmethane sulfonate and the computed linear regression; data were taken from the report of an experiment by Konzak et al. (*16*) and reproduced from (*15*). Similar data for Atlas-57 barley following the treatment of seeds with either gamma radiation or unmoderated fission neutrons are shown in Figures 2 and 3; data were taken from the literature (*15, 17–19*). These data show conclusively that the barley system will yield mutants following seed treatment with either chemical or physical mutagens and that the response is linear for the range of treatments used in these experiments.

The barley system has also been used in experiments involving exposure of plants throughout their life cycle to various levels of γ-radiation. One such experiment was conducted using Atlas-57 plants grown in the gamma garden at Brookhaven National Laboratory (*20*). The M_0 plants were exposed to γ-radiation for 20 hr/day from the time of emergence to the time of harvesting (a total of 96 days). Exposure rates ranged from 0.17 R/day (16.5 R total ex-

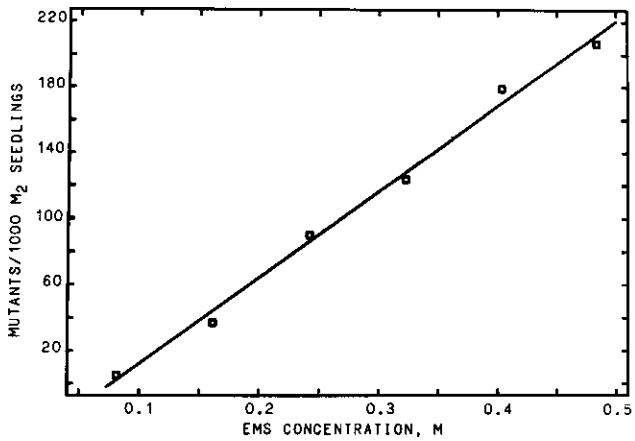

FIGURE 1. Frequency of chlorophyll-deficient phenotypes per 10^3 M_2 seedlings of Himalaya barley as a function of ethyl methanesulfonate concentration used to treat the seed. Figure from Constantin (*15*); data from Konzak et al. (*16*).

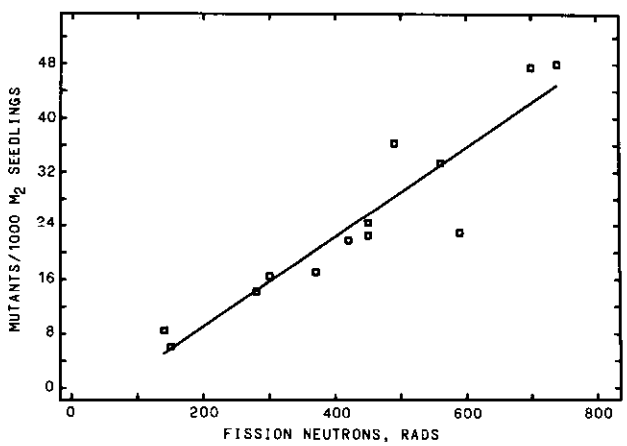

FIGURE 2. Frequency of chlorophyll-deficient phenotypes per 10^3 M_2 seedlings of Atlas-57 barley as a function of ^{60}Co γ-radiation dose to dormant seeds. Figure from Constantin (*15*) and data from Conger and Constantin (*17–19*).

posure) to 23.2 R/day (2240 R total exposure). Five spikes were harvested from each M_1 survivor (2200–3000 M_1 seeds planted per treatment) and planted in greenhouse beds for scoring for chlorophyll-deficient phenotypes. Data are shown in Table 1. Number of mutants per 10^4 seedlings increased in an approximately linear fashion as the exposure per day increased from 0.17 to 23.2 R/day. Even the lowest exposure per day increased the mutant frequency from 1.3 to 4.3 per 10^4 seedlings.

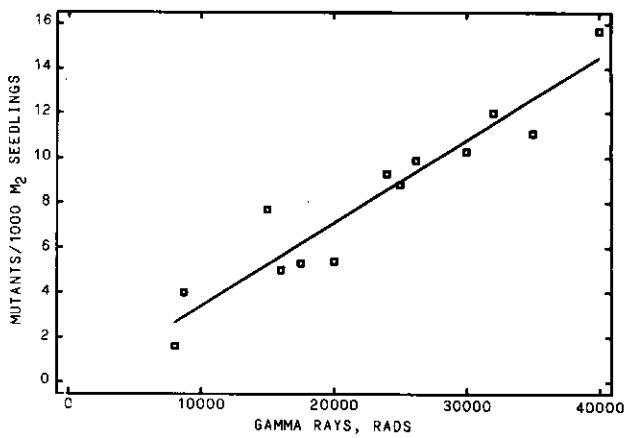

FIGURE 3. Frequency of chlorophyll-deficient phenotypes per 10^3 M_2 seedlings of Atlas-57 barley as a function of unmoderated fission neutron dose to dormant seeds. Figure from Constantin (*15*) and data from Conger and Constantin (*17–19*).

Table 1. Mean number of chlorophyll-deficient phenotypes per 10^4 M_2 seedlings relative to γ-radiation exposure during the life cycle of the plant.[a]

Exposure rate, R/day	Exposure, R	Mutants/10^4 M_2 seedlings
0	0	1.3
0.17	16.5	4.3
0.34	32.0	3.9
0.73	69.5	4.5
1.45	139.2	4.1
3.17	303.7	7.0
7.15	692.3	24.9
12.76	1235.0	23.3
23.20	2240.0	45.4

[a] Data of Conger et al. (*20*).

The Yg_2 System in Maize

The Organism. Maize (*Zea mays*) is a diploid with $2N = 10$ chromosomes. The plant is a monoecious summer annual that is mostly cross-fertilized by wind-dispersed pollen. Because the plant has separate pistillate and staminate inflorescences, both selfing and hybridizing can be achieved readily through the use of appropriate bags. Maize has been used extensively in genetic studies; thus numerous loci have been identified and assigned to specific chromosomes. For more details refer to Neuffer and Coe (*9*).

The System. The Yg_2 locus is found near the end of chromosome 9. Leaf color is normal (green) in the homozygous dominant and in the heterozygote but is abnormal (yellowish green) in the homozygous recessive. The loss (deletion) or alteration of function (mutation) of the dominant allele in

the heterozygote results in the yellowish green phenotype. When seeds or young seedlings are exposed to mutagenic agents that induce either a deletion or mutation of the dominant allele, yellowish green sectors appear on the leaves. The younger the leaf primordium is at the time of treatment, the larger the sectors are; conversely, the older the leaf primordium is, the smaller the sectors are. Older leaves have more sectors than do younger leaves. The mature maize embryo in the dormant kernel possesses six leaf primordia. For more details refer to Stein and Steffensen (*21*) and Smith et al. (*22*).

Procedures. Generally, seeds heterozygous for the Yg_2 locus are treated with a mutagenic agent, and plants are grown for approximately one month. The frequency of yellowish green sectors is then determined for leaves 3, 4, 5, or 6. Growing the plants at a relatively cool (60–75°F) temperature helps in the identification of sectors.

Results. The first results are from an experiment by Smith et al. (*23*) in which dry seeds (6.7% water content) were exposed to 250 kVp x-rays. The x-ray doses, the number of leaves scored, and the mean frequency of sectors per leaf 4 are shown in Table 2. The relationship between x-ray dose and mean frequency of sectors on leaf 4 was linear. No relationship was detected between dose rate (455 to 980 rad/min) and the frequency of yg_2 sectors. Likewise, no post-irradiation storage (up to 14 days) effect was detected.

The second results are from an experiment by Conger (*24*) in which soaked seeds were irradiated with either ^{60}Co γ-rays or unmoderated fission neutrons. Dose of γ-rays and fission neutrons, number of leaves scored, and mean number of sectors per leaf 4 are shown in Table 3. Soaking the seeds for 96 hr prior to irradiation allowed the expansion of existing leaf primordia, but no new organs were initiated. Cytophotometric studies revealed that 33.2, 16.4, and 39.5% of the cell population scored (shoot apex and leaves 4, 5, and 6) were in G_1, S, and G_2, respectively. In comparison, dry seeds had a greater fraction of the cell population in G_1 (*24*). In the case of the presoaked seeds, a log-log relationship existed between radiation dose and the mean frequency of sectors per leaf 4.

Table 2. Mean frequency of yg_2 sectors per leaf 4 of heterozygous maize plants induced by x-irradiation of dormant seeds.[a]

Dose, rad	Dose rate, rad/min	Mean number of sectors/leaf 4
1,500	455	0.20
1,590	980	0.40
2,000	980	0.32
3,000	455	1.09
3,000	593	1.89
4,000	980	1.82
4,500	455	1.29
6,000	980	2.29
6,000	455	4.03
6,000	593	5.01
7,500	455	3.29
7,650	980	5.43
9,000	455	4.20
9,000	593	7.02
15,600	980	11.89

[a] Data of Smith et al. (*23*).

Table 3. Mean number of yg_2 sectors per leaf 4 of heterozygous maize plants as affected by dose of γ radiation and fission neutrons to presoaked seeds.[a]

Exposure	Dose, rad	Sectors per leaf (mean ± S.E.)
None	0	0.00 ± 0.00
Fission neutrons	0.5	0.13 ± 0.06
	1.0	0.23 ± 0.08
	2.0	0.68 ± 0.16
	4.0	0.78 ± 0.12
	7.0	0.97 ± 0.23
	16.0	2.03 ± 0.36
	23.0	3.56 ± 0.39
	32.0	5.25 ± 0.50
γ-Radiation	30	0.13 ± 0.04
	60	0.39 ± 0.12
	120	0.83 ± 0.12
	240	2.60 ± 0.33
	360	4.57 ± 0.47
	480	6.90 ± 0.58
	600	9.63 ± 0.53

[a] Data of Conger (*24*).

In this system the yg_2 sectors are generally assumed to be the result of a chromosomal deletion involving the short arm of chromosome 9. This assumption is further supported by the results when ethylmethane sulfonate is used compared to those when sodium azide is used to treat seeds (*25*). Ethyl methanesulfonate can cause chromosomal aberrations, whereas sodium azide does not. In the Yg_2 system, ethyl methanesulfonate induces a high frequency of sectors, whereas sodium azide induces a low frequency.

The Thiamine Auxotrophy System in *Arabidopsis*

The organism. *Arabidopsis thaliana,* a short life cycle (≃ 30 days) member of the *Crucifereae* family, is a self-fertilized diploid with $2N = 10$ chromosomes. *Arabidopsis* has been used as the test organism for numerous experiments involving mutagenesis. The plant offers several advantages including a number of marker loci, a complete life cycle requiring from 30 to 50 days, a small size permitting large numbers of plants to be grown in a

relatively small space (in fact, the plant can be grown to maturity in a test tube), and production of numerous seeds by each plant. Rédei (*3*) has recently reviewed the literature on *Arabidopsis*.

The System. Auxotrophs for thiamine are isolated from M_2 seedlings on the basis of appearance within 10 days after germination (*26*). The thiamine auxotrophs germinate with green cotyledons and then fail to develop green leaves. A simple counting procedure is then used to determine frequency.

Procedure. Rédei (*27*) has described the procedures used to isolate thiamine auxotrophs and other classes of mutants. Basically, one attempts to handle M_1 seeds in bulk. For example, he suggests the treatment of 10^5 seeds to be suspended in a cold viscose agar solution and dispensed at the rate of 100 seeds per 5 ml of solution per 5-in. pot. The M_1 generation plants are grown under continuous illumination for 6–7 weeks in order to sample all plants. M_2 seed can be harvested in bulk from either all plants or plants within sections of a pot. M_2 seeds are planted at the rate of 100 per 5-in. pot in the same manner as the M_1 generation seeds. The thiamine auxotrophs are identified and staked. They are then fed twice weekly with a dilute solution and thiamine until maturity. Each suspect mutant can then be progeny tested for verification of the mutation. It should be emphasized that other types of mutants such as chlorophyll-deficient phenotypes can be assessed in the same population.

Results. Rédei (*25*) reports that ca. 200 thiamine auxotrophs have been isolated and characterized. He further reports that the frequency of thiamine auxotrophs approximates that observed in *Penicillium*, i.e., 2.5×10^{-4} following mutagenic treatment (*26*).

Role of Specific Locus Systems in Higher Plants to Monitor the Environment of Mutagens

The present concept of testing for mutagenicity involves a tiered-system approach including both prokaryotic and eucaryotic organisms. These organisms are used as surrogates for humans, and any substance which yields positive results is suspect. Plant systems such as *Arabidopsis, Zea, Hordeum*, etc. can serve as another data point in the assessment of mutagenicity. Plant systems offer several advantages including the ability to test the environment *in situ* for the presence of mutagens. The climatic conditions that prevail in the area to be monitored will dictate to some extent the kind of plant that can be used without the benefit of environment-controlled chambers. Plant systems are available that vary from a single specific locus to numerous specific loci that can monitor a site over several days in the case of *Arabidopsis* to several weeks in the case of *Hordeum*.

Plant systems are also available that can be used to assess the mutagenicity of chemical compounds either alone or in combinations in laboratory-type experiments. In addition, some plant systems can be used advantageously to monitor for chromosomal aberrations and toxicity.

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REFERENCES

1. Grant, V. Genetics of Flowering Plants, Columbia Univ. Press, New York, 1975.
2. Rick, C. M. The tomato. In: Handbook of Genetics, Vol. 2, Plants, Plant Viruses and Protists, R. C. King, Ed., Plenum Press, New York, 1974, p. 247.
3. Rédei, G. P. *Arabidopsis thaliana*. In: Handbook of Genetics, Vol. 2, Plants, Plant Viruses and Protists, R. C. King, Ed., Plenum Press, New York, 1974, p. 151.
4. Wilson, K. A method for inducing and isolating biochemical mutants in higher plants. EMS Newsletter 5: 30 (1971).
5. Garner, W. W. The Production of Tobacco. Blakiston, Philadelphia, 1946.
6. Christianson, M. L., and Chiscon, M. O. Use of haploid plants as bioassays for mutagens. Environ. Health Perspect. 27: 77 (1978).
7. Nilan, R. A., and Vig, B. K. Plant test systems for detection of chemical mutagens. In: Chemical Mutagens, Principles and Methods for Their Detection, Vol. 4., A. Hollaender, Ed., Plenum Press, New York, 1976, p. 143.
8. Sparrow, A. H., Underbrink, A. G., and Rossi, H. H. Mutations induced in *Tradescantia* by small doses of x-rays and neutrons: analysis of dose-response curves. Science 176: 916 (1972).
9. Neuffer, M. G., and Coe, E. H., Jr. Corn (maize). In: Handbook of Genetics, Vol. 2, Plants, Plant Viruses and Protists, R. C. King, Ed., Plenum Press, New York, 1974, p. 3.
10. Robinson, R. W., and Whitaker, T. W. *Cucumis*. In: Handbook of Genetics, Vol. 2, Plants, Plant Viruses and Protists, R. C. King, Ed., Plenum Press, New York, 1974, p. 145.
11. Blixt, S. The pea. In: Handbook of Genetics, Vol. 2, Plants, Plant Viruses and Protists, R. C. King, Ed., Plenum Press, New York, 1974, p. 181.
12. Gustafsson, A. The mutation system of the chlorophyll apparatus. Lund University. Arsskr. N. F. Avd. 2. 36(11): 1 (1940).
13. Gustafsson, A. Studies on the experimental control of the mutation process. In: Proceedings of the Radiobiology Symposium, Liege, Academic Press, New York, 1954, p. 282.
14. Wettstein, D. von, et al. The genic control of chloroplast development in barley. In: Autonomy and Biogenesis of Mitochondria and Chloroplasts. N. K. Boardman, A. W. Linnane, and R. M. Smillie, Eds., Amsterdam, 1971, p. 205.
15. Constantin, M. J. Mutations for chlorophyll-deficiency in barley: Comparative effects of physical and chemical mutagens. In: Barley Genetics, III, H. Gaul, Ed., Karl Thiemig, Munich, 1976, p. 96.
16. Konzak, C. F., et al. Efficient chemical mutagenesis. In: The Use of Induced Mutations in Plant Breeding, FAO/IAEA

Tech. Mtg., Rome (Radiation Bot. 5), Pergamon Press, Oxford, 1965, p. 49.
17. Conger, B. V., and Constantin, M. J. The effectiveness of fission neutrons, 14.7 MeV monoenergetic neutrons and ^{60}Co gamma radiation on seedling growth reduction and induction of chlorophyll-deficient mutations in barley. In: Biological Effects of Neutron Irradiation (Proc. IAEA Symp., Neuherberg), IAEA, Vienna, 1974, p. 417.
18. Constantin, M. J., et al. Chlorophyll-deficient mutants in barley: effects of chemical mutagens on irradiated and non-irradiated seeds after various periods of pre-soaking. In: Polyploidy and Induced Mutations in Plant Breeding, Proc. Mtgs. FAO/IAEA and EUCARPIA, Bari, Italy, IAEA, Vienna, 1974, p. 53.
19. Constantin, M. J., and Conger, B. V. Comparative mutagenicity of beta, fission neutron, and gamma-radiation in barley. Radiat. Res. 59(1): 154 (abstr.) (1974).
20. Conger, B. V., Constantin, M. J., and Bottino, P. J. Chlorophyll-deficient mutation frequency in barley following continuous gamma irradiation over a wide range of exposures throughout the life cycle. Mutat. Res. 23: 327 (1974).
21. Stein, O. L., and Steffensen, D. Radiation-induced genetic markers in the study of leaf growth in *Zea*. Amer. J. Bot. 46: 485 (1959).
22. Smith, H. H., et al. The deuteron microbeam as a tool in botanical research. Radiat. Bot. 1: 255 (1962).
23. Smith, H. H., et al. RBE of monoenergetic fast neutrons: cytogenetic effects in maize. In: Biological Effects of Neutron and Proton Irradiations, IAEA, Vienna, 1964, p. 233.
24. Conger, B. V. Effectiveness of fission neutrons versus gamma radiation for inducing somatic mutations in presoaked seeds of maize. Mutat. Res. 34: 223 (1976).
25. Conger, B. V., and Carabia, J. V. Mutagenic effectiveness and efficiency of sodium azide versus ethyl methanesulfonate in maize: induction of somatic mutations at the yg_2 locus by treatment of seeds differing in metabolic state and cell population. Mutat. Res. 46: 285 (1977).
26. Rédei, G. P. Induction of auxotrophic mutations in plants. In: Genetic Manipulations with Plant Material, NATO Advanced Study Institute, Liege, Belgium, Plenum Press, New York, 1976, p. 329.
27. Rédei, G. P., and Acedo, G. Biochemical mutants in higher plants. In: Cell Genetics in Higher Plants, D. Dudits, G. L. Farkas, and P. Maliga, Eds., Akademiai Kiado, Budapest, 1976, p. 39.